

Effects of magnetic water and feeding rate on growth performance and immunity of Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Objective: The present study was designed to investigate the effect of magnetic water treatment technique and different feeding rates on growth performance, feed utilization, water quality parameters, chemical composition and intestinal histomorphometric parameters of monosex Nile tilapia (*Oreochromis niloticus*).

Design: Experimental study with a factorial design.

Fish: A total of 2880 apparently healthy monosex Nile tilapia (*O. niloticus*) with an average initial body weight of 69.86 ± 0.8 g were randomly distributed into 18 concrete ponds.

Procedures: Experimental fish were fed on commercial diet (30.1% protein and 4600 Kcal GE/kg diet) based on three levels of feeding rate 3, 4 and 5% from fish biomass for both treated and control groups. Water was treated with magnetic waves at 0.2 Tesla (Tesla= 2000 Gauss) compared to the control group water (zero Tesla). Growth performance parameters, feed utilization, chemical composition and intestinal morphometric analysis were calculated in all groups at the end of the experiment after eight weeks.

Results: The results indicated that growth performance, feed utilization and intestinal histomorphometric analyses improved significantly ($P < 0.01$) in magnetic water groups compared to control groups at the three levels of feeding rate. In addition, water physicochemical parameters including Ammonia (NH_4), Nitrate (NO_3), Nitrite (NO_2), PH and dissolved oxygen (DO) significantly improved in magnetic water treated groups at the three feeding rate levels.

Conclusions and clinical relevance: In conclusion, magnetic treatment of water could improve water quality parameters, fish growth performance, feed utilization, and intestinal histomorphometric analyses at different feeding rate.

Keyword: Magnetic water, feeding rate, Nile tilapia, growth performance and intestinal morphometrics.

1. INTRODUCTION

Water is a paramagnetic compound with small and positive susceptibility to magnetic fields through them water pass in a multi-reversing polarity magnetic field (MF). The dipolar movements of the molecules of dissolved solids and water molecules are affected in such a way that at the instant of crystal formation, the crystal form is divided into thin layers and the ions align according to a single magnetic axis[1]. Magnetic water treatment (MWT) is a simple and efficient approach where the water flows through a (MF) or combination of magnetic fields. It is a nonchemical treatment of water that does not require any filtration substitutes. The biological technique using MF to purify water is considered as a simple simulation of what happens in nature, as when water is subjected to a MF field and as a result, becomes more biologically active [2].

In non-magnetic treated water (MTW), the molecule clusters and loosely attracted, this loose and chaotic form of attraction predisposes the water to toxins and pollutants to travel inside the water molecule cluster. The large structure of these water molecule clusters, or presence of toxins blocks

large portions of these clusters when they pass through the cell membrane. The smaller size of these chaotic clusters, some of them carrying toxins, can enter the cell with consequent harmful effects [3]. While, exposing water to MF affects water physicochemical properties and have positive effects on the biological activity of organism that consume such treated water [4]. Furthermore, MWT stimulating the activity of proteins, enzymes, the movement of free radicals and enhancing the overall biochemical processes inside the living cells [5].

Magnetic biological technology offers several advantages over traditional chemical treatments and has been shown to improve growth rates and reduce the mortality rate [6]. [7] reported the enhancing effect of exposure to MF on growth, immune and digestive enzyme levels in juvenile Sea cucumbers *Apostichopus japonicus*. Furthermore, [8]; [9];[10] ; [11] and [12] have evaluated the effect of MF treated water on improving water quality, growth parameters, feed utilization and immunity. However, the field application of the magnetic treatment in tilapia reared under different feeding rates still new approaches and need further research. Therefore, the main aim of the present study was to evaluate the effect of

MWT under different feeding rate and how it affects water parameters, fish growth performance, feed utilization, body composition, and intestinal histomorphometric parameters of Nile tilapia (*Oreochromis niloticus*) reared in concrete ponds for 8 weeks.

2. MATERIALS AND METHODS

2.1. Fish and experimental design

Apparently, healthy monosex Nile tilapia (*O. niloticus*) with an average initial weight of 69.86 ± 0.8 g were purchased from a commercial hatchery, Sharkia Governorate, Egypt. Fish were transported alive to the fish experimental farm, Department of fish production, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. A total number of 2880 fish were randomly distributed into 18 concrete ponds ($1 \times 4 \times 1$ m with 4 m^3 of water for each) in three groups (3 replicates each group). Each pond was filled with water up to 100 cm and the level was maintained throughout the experimental period. The experiment was based on a 2×3 factorial design with three feeding rates; (3, 4 and 5% feeding rate), and stocking density were 40 fish/ m^3 in all ponds. The study lasted for 8 weeks after two weeks' acclimation period, all experimental ponds were supplied with dechlorinated tap water through a water pipeline system and were supplied with air through air pipeline using air blower 2 HP, Italy. The water was renewed at a rate of 30% every 24 hours. Fecal matter and feed wastes were removed everyday by syphoning

Two types of water were used, normal water (NW) and MTW with magnet strength of 2000 Gauss (0.2 Tesla), Delta water Co. for water treatment (Japan) as shown in figure 1. This device is composed of an inner magnet surrounded by a copper housing and an outer magnet protected by a steel shield from outside with in and out one-way openings for current water. The proximate analyses of diet and fish samples were taken randomly at the beginning and after 2 months at the end of the trial for whole body analyses including moisture, crude protein, crude fat, crude fiber and total ash content were determined according to Association of Official Analysis Chemists [13] methods. These samples were kept frozen at 4°C till the time of analysis [14].

2.2. Water quality parameters

Water temperature was measured in each pond daily, dissolved oxygen was measured directly by using oxygen-thermometer apparatus (XSI model 58, Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). Ammonia, Nitrate, Nitrite and pH were measured by Hanna Instrument 83205 Boiler and cooling tower photometer, USA. Ammonia ($\text{NH}_4\text{-N}$ mg/L) was measured using Ammonia MR reagent Hi 93715-01. Nitrate ($\text{NO}_3\text{-N}$ mg/L) was measured using Nitrate reagent Hi 93728-01. Nitrite ($\text{NO}_2\text{-N}$ mg/L) was measured using Nitrite reagent Hi 93708-01. Hydrogen ion (pH) was measured using pH reagent Hi 937110-01.

2.3. Experimental diet

All fish groups were fed on commercial diet, which consisted of fishmeal 18.0%, soybean meal 29.0%, yellow corn 20.0%, wheat bran 15.0%, alfalfa hay 12.0%, sunflower oil 3.0%, minerals mixture 1.0%, vitamin mixture 1.0% and carboxymethyl cellulose 1.0%. The chemical composition of the diet was crude protein 30.11%, ether extract 12.35%, Ash 14.34%, nitrogen free extract 43.20 and gross energy 4600 Kcal/kg as shown as Table 1. Fish were fed three times at 8.00, 12.00 and 01.00 o'clock. The fish in each pond were weighed biweekly, and the feed weight was adjusted after each fish weighing.

2.4. Growth performance parameters and feed utilization

All fish were separately weighed to the nearest 69.86 ± 0.8 g at the beginning of the experiment and every two weeks intervals throughout the experimental period. The growth performance and feed utilization efficiency were calculated as following:

Weight gain (WG) = final weight (g/fish) – initial weight (g)
 Daily weight gain (DWG) = body weight gain (BWG) / period.
 Feed conversion ratio (FCR) = feed intake (g) / body weight gain (g)
 Feed efficiency ratio (FER) = body weight gain (g) / feed intake (g)

Protein efficiency ratio (PER) = body weight gain (g) / protein intake (g)

2.5. Histomorphometric analyses

For histomorphometric analyses, three fish from each group were randomly sampled. Portion (about 1cm^2) of anterior intestine (located 2cm^2 after the pylorus) [15], it was dissected as it is the main site for absorption [16]. Specimens were washed with saline and preserved in 10% neutral buffered formalin for 24hrs then prepared for histological examination according to Bancroft and [17]. Ascending Graded concentration of ethyl alcohol as dehydrating agent and xylene as clearing agent were used. After dehydration and clearing the specimens were embedded in paraffin wax, cut with rotatory microtome ($5\mu\text{m}$ thickness) and mounted on coated glass slides. Sections were stained with H&E for morphometric evaluation of villi or with a combination of periodic acid-Schiff reagent (PAS) and Alcian blue (AB) [18] for goblet cell count. The histomorphometric analysis were performed using ImageJ program (version 1.36, NH, USA). Five sections from each fish were subjected to the histomorphometric analysis [15] to estimate the villi length (from the villous tip to the bottom) ($10\times$), epithelial thickness and intraepithelial lymphocyte numbers ($40\times$) and goblet cell numbers within the same villi ($10\times$). Mean \pm SE data were measured from different measurements obtained from each sample.

2.6. Statistical analyses

The data were statistically analyzed using SAS software package V.9. [19] according to the following model: $Y_{ijk} = \mu + D_i + M_j + DM_{ij} + e_{ijk}$. Where, μ is the overall mean, D is the fixed effect of feeding rate ($i = 1 \dots 3$), M is the fixed effect of magnetic water ($j = 1 \dots 2$), DM_{ij} is the interaction effect of feeding rate and magnetic water and e_{ijk} is random error. statistically using two-way analysis of variance (ANOVA). Differences between means were determined and compared by using Duncan's multiple range test and considered significant at $p < 0.05$ [20]. Non-normal data (total bacterial count) were compared.

Table 1. Formulation and chemical proximate analysis of the experimental diet.

Ingredients (g/ kg)	
Fish meal	18.0
Soybean meal	29.0
Yellow corn	20.0
Wheat bran	15.0
Alfalfa hay	12.0
Sunflower oil	3.0
Mineral mixture	1.0
Vitamin mixture	1.0
Carboxymethyl cellulose	1.0
Total	100
Chemical composition (g/ kg ⁻¹)	
Crude protein	30.11
Ether extract	12.35
Ash	14.34
Nitrogen free extract (NFE) ¹	43.20
Gross energy (GE) ²	4600 Kcal/kg

¹ NFE, nitrogen free extract = 100 - (CP+ CF+ EE+ Ash %).

² GE, gross energy, calculated according to Jobling (1983), using the 5.65, 9.45, and 4 for CP, EE and NFE, respectively.

3. RESULTS AND DISCUSSION

3.1. Growth performance

After 8 weeks of treatment applied, final weight (FW), weight gain (WG) and daily weight gain (DWG) were affected significantly ($P < 0.001$) with feeding rate. FW, WG and DWG recorded higher values 124.02, 62.98 and 1.12 g/ fish, respectively in 4%feeding rate group as shown in Table 2. As for magnetic effect, groups treated with magnetic water recorded higher significant values ($P < 0.001$) in FW, WG and DWG; 122.01, 60.99 and 1.08 g /fish, respectively. As shown in Table 2 FW, WG and DWG as affected with the interaction between the feeding rate and magnetic water treatment showed that the highest value was recorded in the 4% MW group (127.80, 66.58 and 1.18) g/fish, respectively followed by the 5% MW group (121.73, 60.96 and 1.08) g/fish, respectively. While the groups 3% NW recorded the lowest values.

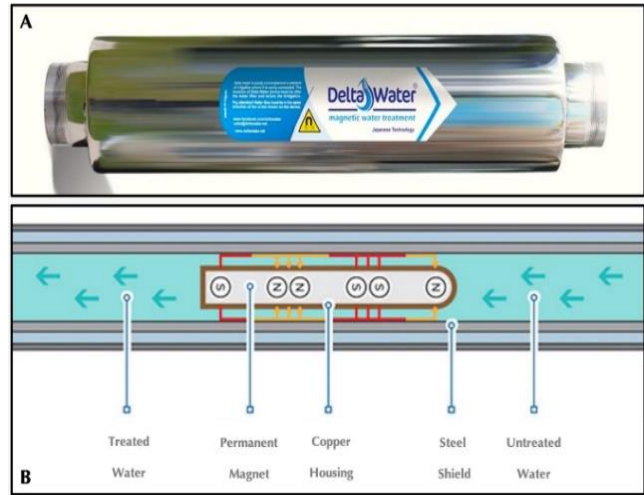


Figure 1. Magnetic water device. Where: (A), a photograph of the whole device, and (B), A schematic diagram of the device showing the inner configuration.

Table 2. Effect of feeding rate, magnetic water, and their interaction on growth performance of Nile tilapia.

Item	IW	FW	WG	DWG
Feeding rate (FR)				
FR 3%	60.76	116.91 ^b	56.15 ^b	1.01 ^b
FR 4%	61.02	124.02 ^a	62.98 ^a	1.12 ^a
FR 5%	60.81	110.73 ^c	49.91 ^c	0.89 ^c
Standard error (SE)	±0.23	±1.09	±1.18	±0.02
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Water type				
Normal water (NW)	60.72	112.43	51.70	0.92
Magnetic water (MW)	61.02	122.01	60.99	1.08
Standard error (SE)	±0.18	±0.8	±0.96	±0.017
Significant	<0.0001	<0.0001	<0.0001	<0.0001
Interaction between feeding rate and type of water				
FR 3%+NW	60.74	112.10 ^{cd}	51.35 ^{cd}	0.91 ^{cd}
FR 4%+NW	60.82	120.20 ^b	59.38 ^b	1.06 ^b
FR 5%+NW	60.61	105.01 ^e	44.38 ^e	0.79 ^e
FR 3%+MW	60.77	121.73 ^b	60.96 ^b	1.08 ^b
FR 4%+MW	61.22	127.80 ^a	66.58 ^a	1.18 ^a
FR 5%+MW	61.01	116.46 ^{bc}	55.45 ^{bc}	0.99 ^{bc}
Standard error (SE)	±0.32	±1.55	±1.67	±0.03
P-value	<0.0001	<0.0001	<0.0001	<0.0001

Means superscripted in the same column with different letters significantly ($P \leq 0.05$) differ.

Initial weight (IW), Final weight (FW), Weight gain (WG), (Daily weight gain (DWG)).

3.2. Feed utilization

feed intake (FI) and protein intake (PI) recorded higher values 124.02, 62.98 and 1.12 g/ fish, respectively in 5% feeding rate group as shown in Table 3. While the groups 3% feeding rate recorded lowest (FI). The 4% FR recorded the lowest FCR value; 1.53 and the 5% feeding rate group recorded the highest value 2.08. It was noticed that PER and FER recorded their highest values 0.65 and 2.17g, respectively

in the 4% feeding rate group. Concerning the effect of water type, (MW) showed significant increase ($P<0.001$) in FI, PI, FER and PER (100.77, 30.23, 0.61 and 2.02g) compared to Normal water (NW). While FCR recorded the best value in (MW)1.66 in comparison with the (NW)1.87.

Table 3. Effect of feeding rate, magnetic water, and their interaction on feed utilization of Nile tilapia.

Item	FI	PI	FCR	FER	PER
Feeding rate (FR)					
FR 3%	94.16 ^c	28.25 ^c	1.68 ^b	0.59 ^b	1.98 ^b
FR 4%	96.66 ^b	29.03 ^b	1.53 ^c	0.65 ^a	2.17 ^a
FR 5%	102.83 ^a	30.85 ^a	2.08 ^a	0.48 ^c	1.61 ^c
Standard error (SE)	±0.66	±0.046	±0.046	0.0113	±0.04
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Water type					
Normal water (NW)	95.03	28.5	1.87	0.54	1.82
Magnetic water (MW)	100.77	30.23	1.66	0.61	2.02
Standard error (SE)	±0.54	±0.16	±0.03	±0.010	±0.03
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction between feeding rate and type of water					
FR 3%+NW	90.33 ^e	27.10 ^d	1.76 ^b	0.56 ^b	1.89 ^b
FR 4%+NW	94.00 ^d	28.20 ^c	1.58 ^{cd}	0.63 ^a	2.10 ^a
FR 5%+NW	100.66 ^b	30.20 ^b	2.27 ^a	0.44 ^d	1.47 ^c
FR 3%+MW	98.00 ^{bc}	29.40 ^b	1.60 ^{bc}	0.62 ^{ab}	2.07 ^a
FR 4%+MW	99.33 ^b	29.80 ^b	1.49 ^{cd}	0.67 ^a	2.23 ^a
FR 5%+MW	105.00 ^a	31.50 ^a	1.89 ^b	0.52 ^{bc}	1.75 ^b
Standard error (SE)	±0.94	±0.28	±0.06	±0.01	±0.06
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means superscripted in the same column with different letters significantly ($P\leq 0.05$) differ.

As shown in Table 3, FI and PI as affected with the interaction between the feeding rate and magnetic water treatment showed that the highest value was recorded in the feeding rate 5% MW group (105.00 and 31.5g respectively), while the lowest record was in feeding rate 3% NW group. As for FCR, feeding rate 4% MW group recorded the lowest value 1.49, while feeding rate 5% NW group recorded the highest value 2.27. FER and PER as affected with the interaction between the feeding rate and type of water, magnetic water treatment showed that the highest value was recorded in the feeding rate 4% MW group (0.67 and 2.23g), respectively while the lowest value was recorded in feeding rate 4% NW group.

These results are in agreement with the findings of previous report [21] whose experiments showed that the fish in magnetic field grew faster than those in non-magnetized water (ordinary water). Also, Tang et al. [7] found that the

effect of magnetic treatment had a positive effect on growth performance and feed utilization on juvenile sea cucumbers.

Table 4. Effect of feeding rate, magnetic water, and their interaction on water quality of Nile tilapia

Item	NH ₄ mg/L	NO ₃ mg/L	NO ₂ mg/L	pH	DO. mg/L
Feeding rate (FR)					
FR 3%	0.015 ^c	0.015 ^c	0.016 ^b	7.58 ^a	6.73 ^a
FR 4%	0.025 ^b	0.027 ^b	0.024 ^b	7.60 ^a	6.03 ^b
FR 5%	0.042 ^a	0.048 ^a	0.051 ^a	7.57 ^a	5.82 ^b
Standard error (SE)	±0.002	±0.002	±0.003	±0.033	±0.143
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Water type					
Normal water (NW)	0.034 ^a	0.031 ^a	0.031 ^a	7.60	5.64 ^b
Magnetic water (MW)	0.021 ^b	0.026 ^b	0.028 ^b	7.56	6.74 ^a
Standard error (SE)	±0.002	±0.002	±0.002	±0.027	±0.117
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction between feeding rate and type of water					
FR 3%+NW	0.013 ^d	0.016 ^c	0.017 ^d	7.61	6.13 ^c
FR 4%+NW	0.031 ^b	0.034 ^c	0.027 ^b	7.61	5.31 ^e
FR 5%+NW	0.051 ^a	0.051 ^a	0.052 ^a	7.58	5.50 ^d
FR 3%+MW	0.011 ^d	0.011 ^f	0.011 ^e	7.54	7.33 ^a
FR 4%+MW	0.017 ^c	0.021 ^d	0.021 ^c	7.58	6.77 ^b
FR 5%+MW	0.032 ^b	0.045 ^b	0.049 ^a	7.56	6.13 ^c
Standard error (SE)	±0.003	±0.003	±0.004	±0.046	±0.202
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means superscripted in the same column with different letters significantly ($P\leq 0.05$) differ.

It has been concluded that magnetized water improved the growth performance of Tilapia and common carp [9, 11,22]. Hassan et al. [10] reported that magnetized water improved growth performances of Jade Perch fish. Results of the current study were in agreement with the findings obtained previously [23] with red hybrid tilapia (*Oreochromis sp.*) in RAS and [24] with rainbow trout (*Oncorhynchus mykiss*). However, the results were in contrast with the findings obtained by Krzemieniewski et al. [25] who found no significant difference between the growth of European sheatfish *Silurus glanis L.* larvae reared in the system modified by the constant magnetic field and the control group.

According to Tyari et al. [26] magnetic water eventually improves the transfer of nutrients to all parts of the body. Irhayyim et al. [11] revealed that the use of magnetized water in the RAS improved the growth performance and feed utilization in common carp. [8] recorded that applying magnetic water on aquaculture, definitely on Nile tilapia farming improved growth performance and feed utilization.

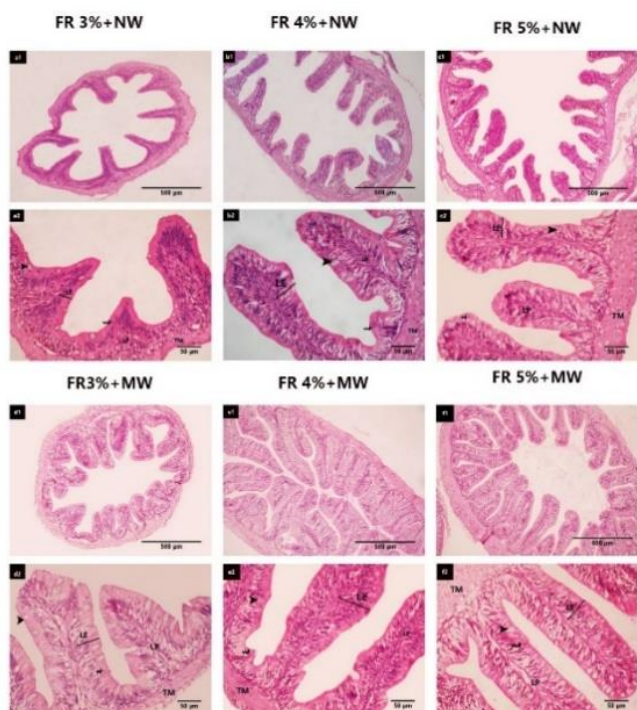


Figure2. Photomicrograph of transverse sections anterior part of intestine stained with H&E showing normal organization of the intestinal wall in all group; longitudinal villi lined with simple columnar epithelium (LE), loose C.T of lamina propria (LP), intact tunica muscularis (TM), intraepithelial lymphocytes (corrugated arrow) and goblet cells (arrow head). (a1, a2) FR3%+NW, a1(x10) and a2(x40). (b1, b2) FR4%+NW, b1(x10) and b2(x40). (c1, c2) FR5%+ NW, c1(x10) and c2(x40). (d1, d2) FR3%+ MW, d1(x10) and d2(x40). (e1, e2) FR4%+MW, e1(x10) and e2(x40). (f1, f2) FR 5%+MW, f1(x10) and f2(x40). FR=feeding ratio, NM=normal water and MW=magnetic water.

As presented in Table 4, averages of ammonia (NH₄), nitrate (NO₃), nitrite (NO₂), hydrogen ion (pH) and dissolved Oxygen (DO) as affected with feeding rate show that NH₄, NO₃ and NO₂ in 5% FR group recorded high significant (P<0.001) values (0.042, 0.048 and 0.051 mg/L, respectively), while their values in 3% FR group were lowest values (0.015, 0.015 and 0.016 mg/L, respectively), As for pH, it didn't record significant differences between all groups. While DO recorded high significant value (P<0.001) 6.73 mg/L in 3% FR group compared to the other groups. Regardless of feeding rate, averages of water quality parameters of NH₄, NO₃, NO₂, pH and DO as affect with the effect of type of water; magnetic water were presented in Table 4. Results of this table reveal that the values of NH₄, NO₃ and NO₂ decreased significantly (P<0.001) in MW group (0.021, 0.026 and 0.028 mg/L, respectively) compared to the NW group (0.034, 0.031 and 0.031 mg/L, respectively). As for pH, there were nonsignificant differences between its values in the MW and NW group, while DO record high significant increase (P<0.001) in MW group 6.74 mg/L compared to NW group 5.64 mg/L.

As presented in Table 4, results of water quality parameters as affected with the interaction between the

feeding rate and the type of water showed that the NH₄, NO₃ and NO₂ recorded low significant decrease in 3% MW groups (0.011mg/L for all), while the highest values were recorded in 5%NW group (0.051, 0.051 and 0.052 mg/L, respectively). As for pH, there were nonsignificant differences between all groups. While DO recorded high significant increase (P<0.001) in MW group with 3, 4 and 5% FR (7.33, 6.77 and 6.13 mg/L, respectively) in comparison with NW group with 3, 4 and 5% FR (6.13, 5.50 and 5.31 mg/L, respectively).

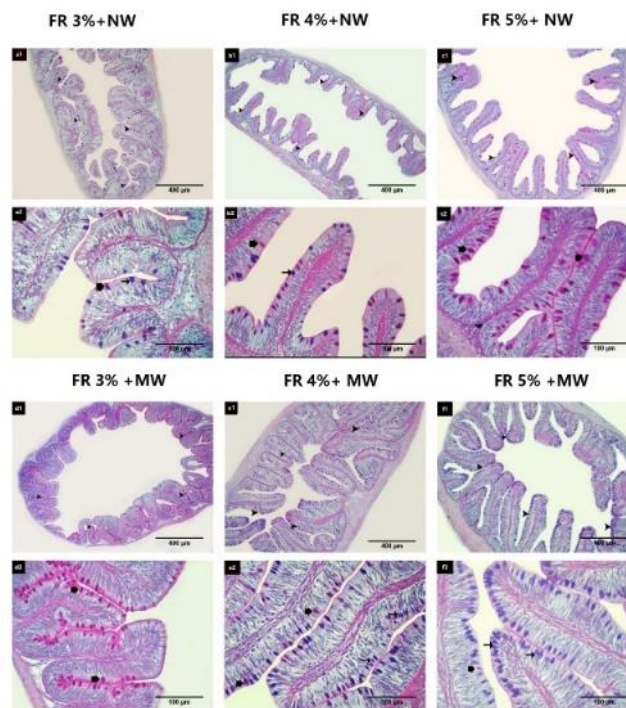


Figure3: Photomicrograph of transverse sections anterior part of intestine stained with Alcian blue PAS stain showing goblet cells density in different group (arrowhead), PAS positive goblet cells (thick arrow) and alcian blue positive goblet cells (arrow). (a1, a2) FR3%+NW, a1(x10) and a2(x40). (b1, b2) FR4%+NW, b1(x10) and b2(x40). (c1, c2) FR5%+ NW, c1(x10) and c2(x40). (d1, d2) FR3%+ MW, d1(x10) and d2(x40). (e1, e2) FR4%+MW, e1(x10) and e2(x40). (f1, f2) FR 5%+MW, f1(x10) and f2(x40). FR=feeding ratio, NM=normal water and MW=magnetic water.

These results agree with the findings obtained [9, 23] who suggested that the ammonium concentration could be reduced by increasing the magnetic intensity. Also, other studies reported that magnets could substantially improve water quality [9, 25, 27]. Krzemieniewski et al. [25] found that increasing the magnetic intensity could reduce ammonia-N in the breeding tank. Nevertheless, reduction in ammonia-N was also observed in wastewater from sewage when a constant magnetic field was used [25]. Alkhazan et al. [28] also showed a significant increase in pH as the magnetic intensity increased. Ahmed et al. [12] recorded that DO and pH values in magnetic water increased as compared to control water, but ammonia was inversely decreased. Similar results were recorded in previous studies [5, 29, 30] pointed that the

increase in magnetic intensity led to an increase in dissolved oxygen concentration compared to normal water. And this ensures that magnetic device improve water quality. The increase of DO may be due to the decrease in organic matter in magnetic water [4]. High pH value probably related to the increase in free carbonate content in water according to the salt dissociate due to magnetic field [31]. The magnetic field increased the free radical formation while the high reactivity and oxidation potential of those chemical compounds may have reduced the concentration of organic matter contained in the analyzed liquids [32]. The lowest value of ammonium may be as the result of oxidizing NH_4 into NO_2 and NO_3 . While the maximum value of NH_4 may be attributed to higher pH and high stock of fish. The results are in agreement with [33] who reported that ammonia concentration was correlated with the amount of stocked fish population. [23] showed significant ($p < 0.05$) increases of the DO and pH.

On the other hand, Irhayyim et al. [11] revealed that the magnetized water had no effects on the concentrations of ammonium nitrogen, nitrite nitrogen and nitrate nitrogen.[10] conducted that there was a slight improvement in water quality parameters for example DO and Ammonia-N were better to manage in magnetized water compared to the control.

Dry matter (DM), crude protein (CP), ether extract (EE), Ash and growth energy (GE) as affected with the feeding rate showed that the highest DM value ($P < 0.001$) was noticed in 5% groups (23.77%) compared the other groups. CP recorded high significant increase ($P < 0.001$) in 3% and 4% groups (71.92 and 71.67%, respectively) compared to 5% group. On the other hand, Ash recorded low significant value in both 3% and 4% groups compared to 5% group. As for EE, GE and DE recorded nonsignificant ($P > 0.001$) between all groups.

Chemical composition as affected with the effect of type of water, MW was presented in Table 5. Results of this table reveal that the values of DM and Ash decreased significantly ($P < 0.001$) in MW group (22.51 and 14.22%, respectively) compared to the NW group (23.1 and 15.62%, respectively). As for CP, it recorded high significant increase ($P < 0.001$) in the MW group (72.01%) compared to the NW group 70.93%. While GE and DE recorded nonsignificant differences ($P > 0.001$) between MW and NW groups.

As presented in Table 5, results of chemical composition as affected with the interaction between the feeding rate and the type of water show that DM recorded significant decrease ($P < 0.001$) in 3% and 4% MW groups (21.97 and 21.73%, respectively) compared to the other groups. As for CP and ether extract, are showed high significant value ($P < 0.001$) in 3, 4 and 5% MW (72.37, 72.83 and 71.37%) and (13.57, 13.77 and 14.03%), respectively compared to 3, 4 and 5% NW groups. While Ash% recorded the highest significant ($P < 0.001$) values in 3, 4 and 5%NW groups (15.03, 16.23 and 16.10%,

respectively). As for GE and DE recorded significant increase in 3, 4, 5 MW and 3% NW compared to the other groups. The results are in accordance with the study of [10], who reported that in magnetic water, the fish were more efficiently converting food into muscle and energy.

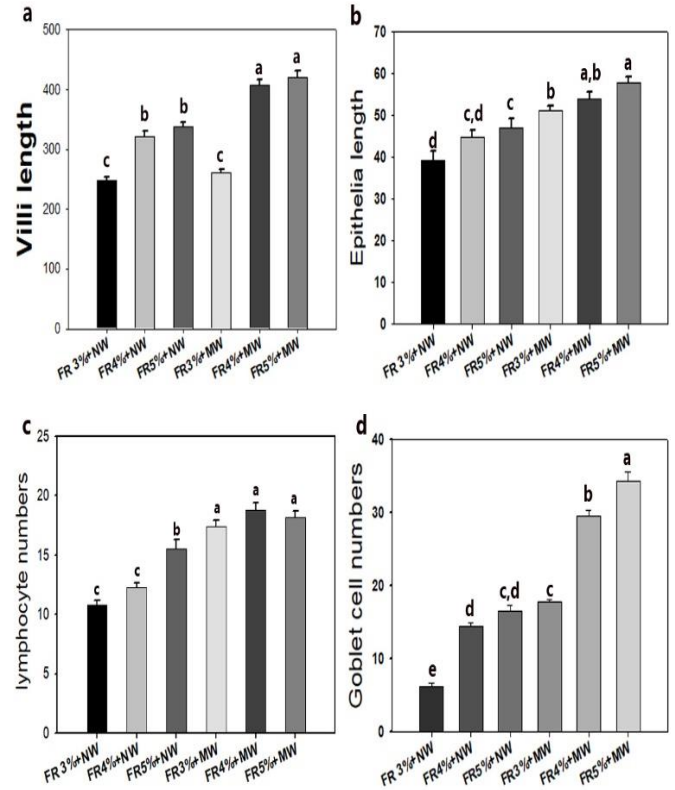


Figure 4. showing, villi length (a), epithelial length (b), lymphocyte numbers (c) and goblet cell numbers (d) in different groups. Data are expressed as Mean \pm SEM. The different letters indicate a significant difference ($P < 0.01$) between experimental groups.

3.3. Histomorphometric results

The histological organization of fish digestive system has been well documented [34], where histological examination of it provide a good indicator for the fish nutritional status [35]. At the same time, any histological variations or alterations in it are important in the nutritional development research [32]. Animal health and nutritional efficiency of the diet mainly depended on the intestine. For this reason, in the current study we examined different histological parameters related to the anterior intestine. To our knowledge, the effects of magnetic water on normal intestinal mucosa haven't been studied previously in the Nile tilapia fish.

In the current study, light microscopic examination of stained intestinal tissues revealed that the intestinal wall in all groups (Fig.2) appeared with normal histoarchitecture that composed of the mucosa, lamina propria-submucosa, tunica muscularis and outermost serosa. The mucosal surface was

arranged into longitudinal folds called intestinal villi. The villi were lined with simple columnar absorptive epithelium interspersed with mucous secreting goblet cells which appeared as vacuoles with H&E stain (Fig. 2), or blue stained with Alcian blue or magenta color with PAS (Fig. 3). An intraepithelial lymphocyte was scattered in the epithelial lining of the villi. The thin connective tissue layer of lamina propria-submucosa extended into the core of each villus. The tunica muscularis is arranged as an inner circular and outer longitudinal layer of smooth muscle. Intestine villi length, epithelial length, intraepithelial lymphocytes, and goblet cells numbers were affected by dietary treatments and water types in both conditions (Fig.2, 3, and 4) All these parameters were significantly increase ($P < 0.05$) in group with feed ratio 4% and 5% compared to group with feed ratio 3% as well as they also significantly increase ($P < 0.05$) in magnetic water compared to normal water. The highest villi (Fig.4a) and epithelial lengths (Fig.4, b) were found in groups with feed ratio 4% and 5% in magnetic water. The increase in intestinal villi and epithelial length leading to enhance the intestinal absorptive capacity for nutrients accompanied with growth performance improvement and better fish health [15, 36]. The mucosal lymphoid tissue of the gut in fish is different from mammals as the fish lacks antigen transporting M cells and Peyer's patches. Normally, it is composed of organized macrophages, granulocytes and lymphoid cells [15]. Intraepithelial lymphocytes, the component of gut associated lymphoid tissue, have a critical role in mucosal defense against any invaded intraluminal antigen [37]. The histological survey in the present study revealed that the number of intra epithelial lymphocytes was significantly higher in the magnetic water than normal water (Fig.4c). The higher number of goblet cells was recorded in the magnetic water group compared to the normal water group (Fig.3 and 4d). The mucus produced by goblet cells play an important role in both physical and chemical defensive barriers, because it removes and traps pathogens, preventing their attachment to the intestinal epithelium. Furthermore, the mucin components such as glycoprotein and mucopolysaccharides also contains considerable amounts of anti-bacterial substances [38, 39] as well as protect and lubricate the intestinal lining [40]. Higher abundance of intraepithelial lymphocytes and goblet cells in the magnetic water group suggested that the magnetic water enhance the intestinal immune defense. The improvement of the intestinal histomorphometric analyses in the magnetic water group is because the water becomes more vital and biologically active when it passes through the magnetic field [2]. At the same time it enhances the movement of the blood to different body's tissues and cells [41] resulting in stimulating the capabilities of the immune system. The magnetization of the water also removes a lot of chemical contaminants and germ and improves the digestive tract function[2].

Conclusion

The results from the present study indicated that growth performance, feed utilization, water quality and intestinal histomorphometry analyses of the MWT showed significant improvement compared to NW with different FR levels. The present study also recommends that using of magnetic water generally improved the production of Nile tilapia.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethical approval

The current study complies with national and international guidelines. The current study was approved by the research ethics committee at Mansoura university Code No: R/78.

Authors' contribution

All authors are equally contributed.

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